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Effect of mushroom diet on pharmacokinetics of gabapentin in healthy Chinese subjects

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Gabapentin and ergothioneine are substrates of OCTN1.
- Shiitake mushrooms contain high amounts of ergothioneine. Ergothioneine's uptake into cells is dependent on OCTN1 and it *trans*-stimulates the secretion of gabapentin *in vitro*. This remains to be established clinically.

WHAT THIS STUDY ADDS

- Ingestion of shiitake mushrooms produced significant increases in plasma ergothioneine concentrations that were sustained for more than 48 h.
- The present study provides clinical evidence of a statistically significant but modest increase in CL_R of gabapentin in Chinese subjects after intake of a high mushroom diet. However, the AUC(0,t_{last}) of gabapentin was not significantly altered.

AIMS

This study evaluated the pharmacokinetics of gabapentin in Chinese subjects who received a diet rich in shiitake mushrooms. Shiitake mushrooms have been shown to contain high amount of ergothioneine. *In vitro* studies have shown that OCTN1-mediated secretion of gabapentin is *trans*-stimulated by ergothioneine. This study also investigated the concentrations of ergothioneine in plasma at baseline and following mushroom consumption.

METHODS

Ten healthy male subjects were recruited and received a diet containing no mushrooms (treatment A) or a high mushroom diet (treatment B; after at least a 7 day washout period) 1 day prior to administration of a single oral dose of gabapentin 600 mg.

RESULTS

Ingestion of shiitake mushrooms produced significant increases in plasma ergothioneine concentrations that were sustained for more than 48 h. A statistically significant but modest increase in the renal clearance (CL_R) of gabapentin occurred after intake of the mushroom diet (91.1 ± 25.1 *vs.* 76.9 ± 20.6 ml min^{-1}, $P = 0.031$). No significant changes in AUC(0,t_{last}) of gabapentin were observed ($P = 0.726$). Creatinine clearance did not correlate with CL_R of gabapentin at baseline (treatment A). After ingestion of the mushroom diet, creatinine clearance accounted for 65.3% of the variance in CL_R of gabapentin.

CONCLUSIONS

These data suggest that diet–drug pharmacokinetic interactions may occur during co-exposure to gabapentin and mushroom constituents. However, as it does not affect the AUC(0,t_{last}) of gabapentin, it may not have clinically important consequences. Shiitake mushrooms can also be used as a source of ergothioneine for future clinical studies.

Introduction

The anti-epileptic agent gabapentin is used to manage severe neuropathic pain arising from cancer and other conditions, including diabetes and post-herpetic and trigeminal neuralgias [1]. Gabapentin exhibits complex non-linear pharmacokinetics and variable bioavailability due to saturable absorption. Gabapentin is minimally bound to plasma proteins (~3%), is not appreciably metabolized and is excreted unchanged by the kidney [2]. Renal clearance (CL_R) includes components of glomerular filtration and active tubular secretion and exhibits extensive inter-individual variation [2]. The novel organic cation transporter OCTN1, which is encoded by the *SLC22A4* gene, mediates active tubular secretion of gabapentin [3]. In individuals who are homozygous for the 1672 C>T single nucleotide polymorphism (SNP) of *SLC22A4* the secretory pathway is almost abolished [4].

Grundemann *et al*. have reported that the principal substrate of OCTN1 is ergothioneine, a unique anti-oxidant that is abundant in most plants and animals [5]. Ergothioneine is derived from dietary sources and its uptake into cells is dependent on OCTN1. Moreover, under *in vitro* conditions, the presence of ergothioneine on one side of the membrane stimulates transport of the alternate OCTN1 substrate gabapentin on the opposite (*trans*) side of the membrane [3]. Thus, the CL_R of gabapentin in subjects could be enhanced during concurrent exposure to ergothioneine, but this remains to be established.

Clinical grade ergothioneine is not readily available but certain foods, including edible mushrooms such as shiitake, are excellent sources of the anti-oxidant. Meat products (kidney, liver) and some plant products (oat bran, black and red beans) have been shown to contain ergothioneine as well [6–9]. The present study evaluated whether a diet rich in shiitake mushrooms may elicit pharmacokinetic interactions with gabapentin in Chinese subjects living in Singapore. This study also investigated the concentrations of ergothioneine in plasma at baseline and following the consumption of mushrooms.

Methods

Subjects

This study was conducted at the Investigational Medicine Unit (IMU) of the National University Health System, Singapore. Ten healthy Singaporean Chinese male subjects between the ages of 27 and 46 years were recruited for the study. Ethnicity was based on the information recorded on the Singapore's National Registration Identity Card. Demographic factors (mean ± SD or median [minimum–maximum]) included age, weight, height and body mass index (BMI) and were 36.5 ± 6.9 years, 71.2 ± 10.6 kg, 174 (158–179) cm and 24 ± 2.5 kg m^{-2}, respectively. Subjects were not taking any regular medications, had normal renal function, haemoglobin >12 g dl^{-1} and a total body weight > 50 kg. There were no clinically relevant abnormalities identified by a detailed medical history, full physical examination, blood pressure, pulse rate measurement, 12-lead ECG and clinical laboratory tests. Subjects were excluded from participation if they were taking a medication that could confound outcomes, had undergone allogeneic bone marrow transplant or had any conditions that may affect drug absorption.

Study design

The study was a single centre, open label, two period, fixed sequence, food–drug interaction study conducted in 10 healthy adult males who met the inclusion and exclusion criteria. A crossover study design was not possible because the half-life of ergothioneine is long, approximately 1 month, based on that reported in rats [10]. Ethics approval from the Domain Specific Review Board (Reference no C/10/207) and regulatory approval from the Health Sciences Authority (Clinical Trial Certificate no CTC1000249) were obtained and compliance with the Singaporean Guideline for Good Clinical Practice was ensured. All subjects had provided written informed consent before any study-related procedures were conducted. Subjects had plasma ergothioneine concentration measured and *SLC22A4* 1672 CC genotype determined during screening. Subjects were admitted to the IMU on day −1, which was at least 24 h prior to dosing on day 1.

Subjects received the following treatments: treatment A: a standardized control diet containing no mushrooms (three meals 1 day prior to gabapentin administration [day −1]) followed by a single oral dose of gabapentin 600 mg next morning (day 1) or treatment B: a high mushroom diet (250 g of mushrooms at each of the three meals 1 day prior to gabapentin administration [day −1]) followed by a single dose of gabapentin 600 mg next morning (day 1). Subjects were fasted for at least 10 h before gabapentin dosing and remained fasted for 4 h post-dose. Control and mushroom meals were prepared by the IMU food provider, with fresh mushroom weighed at each meal. The ergothioneine content in these mushrooms was assayed and quantified to be 264 ppm. There was a washout period of at least 7 days between treatments.

Subjects were required to stay in the IMU for 3 nights per study period for the collection of plasma and urine samples. Pre-treatment blood samples (20 ml) were obtained to serve as a blank for gabapentin and for measurement of plasma ergothioneine. Blood samples (5 ml) were also collected at 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12, 24, 36, 48 h after dosing for gabapentin assay (5 ml each), and at 24 and 48 h after dosing for ergothioneine assay (10 ml) and 12 h for serum creatinine (5 ml). Urinary output was pooled and collected at 0 h (baseline) and at 0–12, 12–24, 24–36, 36–48 h after dosing in both treatments for the assay of gabapentin. The 0–12 h and 12–24 h samples were also used for determination of urinary creatinine and creatinine

clearances at the hospital's laboratory, in accordance with the hospital's standard clinical practice.

SLC22A4 *1672C>T genotyping*

The reference cDNA sequence of *SLC22A4* was obtained from GenBank (http://www.ncbi.nlm.nih.gov, accession no. NM_003059). Primers were obtained from the PharmGKB website which spanned exon 9. Primer sequence used were 5′-CCAACTTCACAAAATGATGCTC-3′ (sense) and 5′-CCCAGCCAACAATATGCTTTAT-3′ (antisense). Direct sequencing of exon 9 of *SLC22A4* was conducted to identify individuals who carried the 1672 C>T SNP, as described previously [11]. All Chinese subjects enrolled in this study carried a homozygous wild-type (CC) genotype.

Determination of gabapentin and ergothioneine

Gabapentin was measured in plasma and urine using a validated liquid chromatography/tandem mass spectrometry (LC-MS/MS) assay [12, 13]. Neurontin® containing 300 mg gabapentin (batch number: 0581110) was purchased from the pharmacy of NUHS. Metformin was obtained from Sigma-Aldrich (St Louis, MO, USA), ammonium formate and high performance liquid chromatography (HPLC) grade acetonitrile were obtained from Merck (Darmstadt, Germany). Formic acid (98–100% v/v) was obtained from Fluka (Germany). Deionized water was obtained from a Milli-Q Plus system (Millipore, Milford, MA, USA).

Chromatography of gabapentin was performed on an Altantis HPLC Silica column (5 μm, 3 mm i.d. × 50 mm) with a Waters Corporation Guard column (Altantis HPLC Silica 3 μm, 2.1 i.d. × 10 mm) and using a mobile phase of 20 mM ammonium formate in acetonitrile (18 : 82, v/v). The pH of the ammonium formate solution was adjusted to 3.2 with formic acid. The HPLC system consisted of an Agilent 1200 series binary pump, online degasser, auto-sampler and thermostatted column compartment (Agilent Technologies, Germany). Mass spectrometry was conducted on an API 3200 Triple-Quadrupole Mass Spectrometer (Applied Biosystems, MDS SCIEX, Ontario, Canada) with Turboionspray source. MS/MS was carried out under positive electrospray ionization and multiple reaction monitoring mode as follows: m/z 172.18 → 154 and 172.18 → 136.93 (qualifying ion) for gabapentin and m/z 130.13 → 71 for metformin. Calibration standards were prepared using 50 μl of saline (0.9% NaCl) spiked with gabapentin working solutions. Final concentrations of calibration standards were 2, 10, 25, 100, 500, 2000, 5000 and 10 000 ng ml^{-1} for plasma and 250, 500, 1000, 2500, 5000 and 10 000 ng ml^{-1} for urine. Under these conditions linearity was demonstrated [coefficient of determination (r^2) > 0.9995]. Interday precision and accuracy in plasma were in the range 1.8–14.4% and 98.3–107.5%, respectively. Intraday precision and accuracy in plasma were in the range 1.1–4.1% and 90.4–104.4%, respectively. Interday precision and accuracy of the method for urine were in the range 3.9–7.1% and 92–99%, respectively. Intraday precision and accuracy in urine were in the range 1.1–4.8% and 85.8–107.4%, respectively. The limit of quantification for gabapentin was 50ng ml^{-1}.

Plasma ergothioneine was measured using a validated LC-MS/MS assay with L-ergothioneine-d_9 as the internal standard (Toronto Research Chemicals Inc., North York, Ontario, Canada). Protein precipitation by acetonitrile was utilized for sample preparation prior to analysis. Chromatographic separation of L-ergothioneine was conducted using gradient elution on Alltima C18 (150 mm × 2.1 mm, 5 μ). The run time was 6 min at a constant flow rate of 0.45 ml min^{-1}. The mass spectrometer was operated in the positive electrospray ionization and multiple reaction monitoring modes. The mass transitions of L-ergothioneine and L-ergothioneine-d_9 were m/z 230 > 127 and m/z 239 > 127, respectively. Excellent linearity [coefficient of determination (r^2) ≥ 0.9998] was achieved for determination of L-ergothioneine in the range of 10–10 000 ng ml^{-1}. Inter-day precision and accuracy of the method were found in the range of 2.4–3.8% and 99.4–104.5% respectively. Intraday precision and accuracy of the method were found in the range of 0.9–3.9% and 94.5–98.2% respectively. The limit of detection for ergothioneine was 10 ng ml^{-1} [14].

Pharmacokinetic analysis

Plasma pharmacokinetic parameters (C_{max}, t_{max}, AUC(0,t_{last}), $t_{1/2}$, CL/*F*) and urinary excretion parameters (A_e, CL_R) for gabapentin and creatinine clearance (CL_{cr}) were determined using non-compartmental methods (Phoenix WinNonlin, version 6.2.1, Pharsight Corporation, Mountain View, CA). Plasma concentration–time data for gabapentin were tabulated and graphically displayed for each subject. AUC(0,t_{last}) was calculated by the linear up/log down method. The terminal elimination rate constant (k_{el}), as the negative slope of the linear portion of the natural logarithmic concentration *vs*. time curve, was used to calculate $t_{1/2}$. The apparent total clearance of gabapentin (CL/*F*) was calculated as the ratio between dose and the AUC(0,t_{last}). Urine samples were analyzed for creatinine and CL_{cr} was estimated by dividing the 24 h excretion rate by the plasma creatinine concentration at midpoint.

Statistical analysis

Data reported by Urban *et al*. were taken as the reference for the mean and standard error of renal gabapentin clearance [3]. Assuming a correlation coefficient of 0.5 between treatment A and treatment B measurements, the estimated SD of the differences is equal to the SD of the individual measurements. Based on the estimated SD of differences in gabapentin CL_R and hypothesized difference of 30% in the mean gabapentin CL_R values between treatments, 10 subjects in each treatment were adequate to

achieve >80% power with 5% significance. Shapiro–Wilk testing established whether continuous data were normally distributed. Parametric and non-parametric tests were then applied when data were normally distributed and not normally distributed, respectively. Statistical comparisons of parameters within groups (treatments A *vs.* treatment B) were made using the parametric paired Student's *t*-test or the non-parametric Wilcoxon signed-rank test, as appropriate. For a more appropriate measure of central tendency, the data that are not normally distributed are expressed as median (range) while the normally distributed data are expressed as mean ± SD. In multiple linear regression analysis variable selection was performed using an automated backward stepwise regression with a *P* value for entry of 0.05 and a *P* value for removal of 0.1, followed by a forward stepwise regression to determine the best independent predictors of the dependent variable. A *P* value < 0.05 was considered to be statistically significant. All analyses were performed using SPSS (SPSS Inc., Chicago, Ill, USA, version 19.0).

Results

Ergothioneine was readily detected in plasma from Chinese subjects who received the control diet (treatment A, Table 1), but not significantly different compared with their respective plasma ergothioneine concentrations during screening ($P = 0.515$, data not shown). Ingestion of the ergothioneine-containing diet produced significant increases in ergothioneine in plasma ($P < 0.01$; Table 1). The increase in ergothioneine concentrations induced by the mushroom-containing diet was sustained over the duration of sampling.

The mean plasma gabapentin concentrations at corresponding times in treatment A and treatment B are shown in Figure 1. As shown in Table 2, intake of the shiitake mushroom diet over 24 h increased the renal clearance (CL_R) of gabapentin by 19% (91.1 ± 25.1 *vs.* 76.9 ± 20.6 ml min^{-1}, $P = 0.031$). Corresponding decreases in the C_{max} of gabapentin (4318 ± 930 *vs.* 5001 ± 1260 ng ml^{-1}), as well as a prolongation of the time to reach maximal plasma concentration (t_{max}) and an increase in total urinary excretion of gabapentin (A_e), were also noted. However, other pharmacokinetic parameters for gabapentin, including the AUC(0,t_{last}), $t_{1/2}$ and CL/*F*, as well as the CL_{cr}, were unchanged by ingestion of the mushroom diet.

From multiple linear regression analysis, no explanatory variables were found to be significant predictors for CL_R of gabapentin in treatment A (control diet). In treatment B (high mushroom diet), CL_{cr} (standardized coefficient = 0.826, $P < 0.01$) was identified as a significant predictor for CL_R of gabapentin, accounting for 65.3% of the variance in CL_R.

Discussion

The present findings demonstrate that a diet containing shiitake mushrooms, as a source of ergothioneine,

Table 1

Plasma ergothioneine concentrations after intake of the control (treatment A) or high mushroom (treatment B) diets at 0, 24 and 48 h after gabapentin dosing

Time after gabapentin dosing (h)	Treatment A (ng ml^{-1})	Treatment B (ng ml^{-1})	Median of differences (95%CI)
0	172 (73–531)	425 (153–1090)	–225.5 (–559, –80)
24	110 (55–353)	234 (125–988)	–138 (–227, –59)
48	110 (54–371)	330 (165–795)	–248.5 (–424, –69)

Data are shown as median (minimum–maximum).

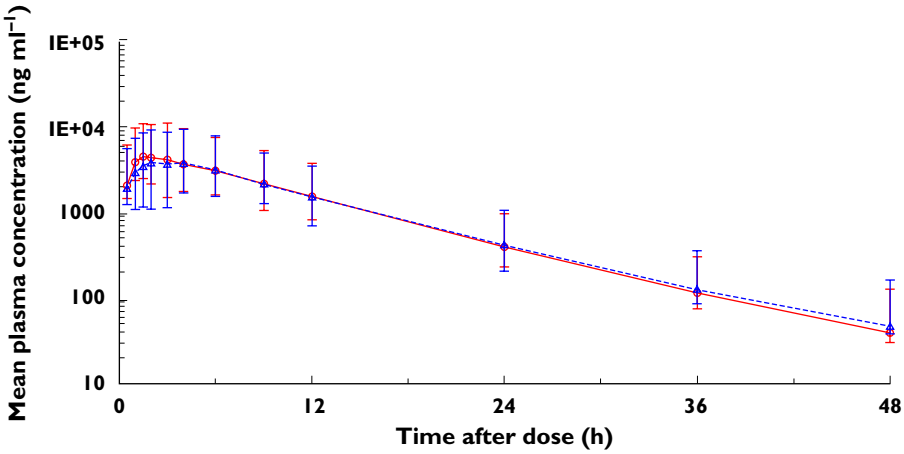

Figure 1

Mean plasma gabapentin concentration–time curves in treatments A and B. —○—, treatment A; —△—, treatment B

Table 2

Pharmacokinetic parameters of gabapentin after intake of the control (treatment A) or high mushroom (treatment B) diets

Pharmacokinetic Parameter	Treatment A (n = 10)	Treatment B (n = 10)	Mean/median of differences (95% CI)
C_{max} (ng ml^{-1})	5001 ± 1260	4318 ± 930**	684 (366, 1001)
t_{max} (h)	1.5 (1–4)	4 (1–6)*	–1.25 (–2.5, 0.0)
AUC(0,t_{last}) (ng ml^{-1} h)	48357 ± 13482	47508 ± 10665	849 (–4468, 6166)
$t_{1/2}$ (h)	6.7 ± 1.3	6.7 ± 1.4	–0.06 (–0.91, 0.80)
CL/F (ml min^{-1})	194.4 (147.5–410.6)	206.7 (158.6–340.3)	–7.02 (–38.44, 82.10)
A_e (mg)	222.4 ± 84.5	253.5 ± 67.6*	–31.1 (–61.7, –0.5)
CL_R (ml min^{-1})	76.9 ± 20.6	91.1 ± 25.1*	–14.2 (–26.8, –1.6)
CL_{cr} (ml min^{-1})	90.6 ± 31.4	105.0 ± 29.8	–14.4 (–42.7, 13.9)

Data are shown as mean ± SD or median (minimum–maximum). A_e, urinary recovery; AUC(0,t_{last}), area under the plasma concentration–time curves from time zero to the last quantifiable sampling point; CI, confidence interval; CL/F, apparent total clearance; CL_{cr}, creatinine clearance; CL_R, renal clearance; C_{max}, observed maximum plasma drug concentration; $t_{1/2}$, half-life; t_{max}, time to reach the C_{max}. *Statistically significant ($P < 0.05$). **Statistically significant ($P < 0.001$).

produces high levels of the anti-oxidant in blood. Urban *et al*. have shown that OCTN1-mediated transport of gabapentin is *trans*-stimulated by ergothioneine *in vitro* [3]. The principal finding to emerge from the present study is that a mushroom diet increased the CL_R of gabapentin ($P = 0.031$). Statistically significant differences were also found between treatments A (control diet) and B (high mushroom diet) in C_{max} ($P = 0.001$), t_{max} ($P = 0.007$) and A_e ($P = 0.047$). Plasma ergothioneine concentrations were readily detected after the high mushroom diet and it is evident that the recovery and CL_R of gabapentin were greater when ergothioneine concentrations were high. A reasonable explanation is that the *in vitro* mechanism of *trans*-stimulation of OCTN1 during the transport of ergothioneine could be relevant *in vivo* and resulted in increased CL_R and excretion of gabapentin. However, since the AUC(0,t_{last}) of gabapentin was not significantly altered, the clinical relevance of a mushroom diet–gabapentin pharmacokinetic interaction may be limited. Nonetheless, this is the first occasion in which a high shiitake mushroom diet has been found to increase and sustain plasma ergothioneine concentrations over 48 h in a clinical study.

It is important to mention that the present study could not rule out the possibility that the measureable plasma ergothioneine concentrations after control diet could have been sufficient to cause near maximal *trans*-stimulation of OCTN1-mediated secretion of gabapentin. However, it may not be feasible to 'wash out' the subjects until the plasma ergothioneine concentrations are undetectable given its long half-life.

The mean values of CL_R of gabapentin obtained in this study (76.9 ml min^{-1}) were lower than those reported by Urban *et al*. (141.4 ml min^{-1}) but the average total urinary excretion of a gabapentin dose was quite comparable (37% *vs*. 45%) [3]. The reasons for these differences remain unclear but may be attributable to intrinsic and/or extrinsic ethnic factors.

Earlier studies reported that CL_{cr} is a significant predictor for gabapentin CL_R, although accounting for a small fraction (23%–33%) [3, 15]. In this study, CL_{cr} was identified as a significant predictor for gabapentin renal clearance in treatment B (high mushroom diet) but not treatment A (control diet). The reasons for this are not clear, but it may be due in part to the size of the population studied.

It is known that gabapentin is absorbed by an active mechanism involving the L-type amino acid transporters and the amino acid transport system $b^{0,+}$, which may also contribute to gabapentin transport [16, 17]. Using the *in situ* single-pass rat model of intestinal perfusion, Nguyen *et al*. demonstrated that dipeptide uptake via PEPT1 trans-stimulated gabapentin uptake via the transport system $b^{0,+}$ and that authentic $b^{0,+}$ substrates, such as the amino acids lysine and arginine, may compete with gabapentin for uptake [16]. Thus, constituents of shiitake mushroom other than ergothioneine, including amino acids, may have the potential to affect the pharmacokinetics of gabapentin. There is a possibility that the observed insignificant change in AUC(0,t_{last}) of gabapentin could be the net effect of the interaction between gabapentin and mushroom constituents at the absorption and disposition sites. Further studies are necessary to test this possibility.

In conclusion, the present study provides clinical evidence of increased CL_R of gabapentin in Chinese subjects, after intake of a high mushroom diet. Thus, diet–drug pharmacokinetic interactions may occur during co-exposure to gabapentin and mushroom constituents including ergothioneine. Nevertheless, it does not affect the AUC(0,t_{last}) of gabapentin and may therefore have limited clinically important consequences. Our findings also document for the first time that ergothioneine concentrations were readily detected and sustained in plasma after the consumption of three meals of 250 g shiitake mushroom. Shiitake mushrooms can be used as a source of ergothioneine for future clinical studies.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organization other than their employer for the submitted work, no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

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